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14. ABSTRACT
We discovered some sequences in the genomes of single celled eukaryotes that resembled incomplete ion channels. Since some of the organisms that have these sequences are responsible for carrying diseases, we wished to investigate whether these sequences coded for viable ion channels. If so, then we hoped that they would be good targets for drug development. We cloned this ion channel from a fungus and studied the channel's expression by injecting it in frog eggs. We noted small currents that had unusual electrical properties but eventually concluded that these currents are artifacts produced by their stimulating an ion current found in frog eggs. We next planned to

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Report Title

Final Report: STIR: Analysis of a Novel Microbial Ion Channel

ABSTRACT

We discovered some sequences in the genomes of single celled eukaryotes that resembled incomplete ion channels. Since some of the organisms that have these sequences are responsible for carrying diseases, we wished to investigate whether these sequences coded for viable ion channels. If so, then we hoped that they would be good targets for drug development. We cloned this ion channel from a fungus and studied the channel's expression by injecting it in frog eggs. We noted small currents that had unusual electrical properties but eventually concluded that these currents are artifacts produced by their stimulating an ion current found in frog eggs. We next planned to test whether these incomplete channels function, instead, as dominant negatives, assembling with and inhibiting complete channels. However, we did not have enough time to complete this aim in the allotted funding period.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

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(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

<u>Received</u> <u>Book</u>

TOTAL:

<u>Received</u> <u>Book Chapter</u>

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Alfredo Ghezzi	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

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FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

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The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

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Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
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Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

INTRODUCTION

We discovered some molecular sequences in the genomes of single celled eukaryotes that resembled incomplete ion channels. Most voltage-gated ion channels have six membrane-spanning helical segments. Two of these segments (S5-S6) form a channel through which ions flow. Another (S4) has numerous evenly spaced positively charged amino acids; the S4 detects membrane voltage and its movement triggers the channel to open. The first three helices (S1-S3) serve as a supporting structure to hold the S4 and prevent it from moving until a voltage stimulus forces it to move. There is a type of ion channel (H+ sensing) that is only S1-S4. This channel passes ions in a novel way. We wondered if the segments of the channel that we discovered (S1-S3) could form a complete ion-passing channel.

Since some of the organisms that have these sequences are responsible for carrying diseases, we wished to investigate whether these sequences coded for viable ion channels. If so, then we hoped that they would be good targets for drug development.

RESULTS

We cloned this ion channel (BdS1-S3) from the fungus Batrachochytrium dendrobatidis (Bd) and studied the channel's expression by injecting it in frog oocytes (eggs). Our controls were oocytes injected with water. We occasionally noted small currents that had unusual electrical behaviors. These currents were not always observed and they did not become more obvious when we incubated eggs for 1,2 or 3 days. These currents activated rapidly to hyperpolarizing stimuli. Repeated stimulation caused greater magnitude currents. We were unable to detect a clear reversal potential for these currents. There was no change in the behavior of the currents when we altered extracellular potassium, sodium or calcium. However, although not as frequently, we saw the same currents with eggs injected with water. Thus, we began to believe that the currents were endogenous to the oocyte rather than reflecting currents from the Bd channel construct. These currents appeared similar to currents described in the literature as an endogenous current found in oocytes that are triggered by the injection of various exogenous substances. Thus, we believe we were not observing currents from the Bd channel.

We then eliminated the S1, S2 or S3 segments from a fruit fly potassium channel (shaker) construct, and switched in the ones from the Bd channel to test whether these segments from Bd could still function. However, this took time and the funding period (9 months) was over before we were able to continue these experiments. We had also planned to inject the Bd channels along with normal shaker channels to test whether the S1-S3 Bd channel acted as a negative dominant rather than a complete channel on its own. If this is so then the more BdS1-S3 that we inject along with a constant amount of shaker RNA, the less shaker current there would be.

Technology Transfer